

## CORRECTED VERSION

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
6 April 2006 (06.04.2006)

PCT

(10) International Publication Number  
**WO 2006/035244 A3**

## (51) International Patent Classification:

A61P 29/00 (2006.01) A61K 31/498 (2006.01)

## (21) International Application Number:

PCT/GB2005/050164

## (22) International Filing Date:

28 September 2005 (28.09.2005)

## (25) Filing Language:

English

## (26) Publication Language:

English

## (30) Priority Data:

60/614,093 28 September 2004 (28.09.2004) US

(71) Applicant (for all designated States except US):  
**PHARMA MAR S.A., SOCIEDAD UNIPERSONAL**  
[ES/ES]; Poligono Industrial La Mina, Avda. de los Reyes,  
1 Colmenar Viejo, E-28770 Madrid (ES).

(71) Applicant (for SD only): **RUFFLES, Graham, Keith**  
[GB/GB]; 66-68 Hills Road, Cambridge, Cambridgeshire  
CB2 1LA (GB).

## (72) Inventors; and

(75) Inventors/Applicants (for US only): **ALLAVENA, Paola**  
[IT/IT]; Istituto di Ricerche Farmacologiche, Mario  
Negri, Via Eritrea, 62, I-20157 Milan (IT). **D'INCALCI,**  
**Maurizio** [IT/IT]; Istituto di Ricerche Farmacologiche,  
Mario Negri, Via Eritrea, 62, I-20157 Milan (IT). **FAIR-**  
**CLOTH, Glynn, THOMAS** [US/US]; Pharma Mar USA,  
Inc., 320 Putnam Avenue, Cambridge, Massachusetts  
02139-4616 (US).

(74) Agent: **Marks & Clerk**; 66-68 Hills Road, Cambridge,  
Cambridgeshire CB2 1LA (GB).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SI, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SI, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

## Published:

— with international search report

## (88) Date of publication of the international search report:

31 August 2006

## (48) Date of publication of this corrected version:

1 March 2007

## (15) Information about Correction:

see PCT Gazette No. 09/2007 of 1 March 2007

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ECTEINASCIDIN COMPOUNDS AS ANTI-INFLAMMATORY AGENTS

(57) Abstract: We have found anti-inflammatory activity in the ecteinascidin compounds. Such compounds have been widely described, and may have the following general formula (I), wherein:  $R^5$  is OH, alkoxy or alkanoyloxy;  $R^6$  is hydrogen, alkyl, alkenyl, alkynyl or aryl;  $R^{12}$  is hydrogen, alkyl, alkenyl, alkynyl or aryl;  $R^{16}$  is hydrogen, alkyl, alkenyl, alkynyl or aryl;  $R^{17}$  is OH, alkoxy or alkanoyloxy;  $R^{18}$  is OH, alkoxy or alkanoyloxy;  $R^{21}$  is H, OH, CN or another nucleophilic group; and  $R^a$  is hydrogen and  $R^b$  is optionally substituted amino, or  $R^a$  with  $R^b$  form a carbonyl function =O, or  $R^a$ ,  $R^b$  and the carbon to which they are attached form a tetrahydroisoquinoline group.



WO 2006/035244 A3

## ECTEINASCIDIN COMPOUNDS AS ANTI-INFLAMMATORY AGENTS

The present invention relates to anti-inflammatory agents. More particularly, the present invention relates to the discovery of anti-inflammatory activity in a known class of compounds.

## BACKGROUND OF THE INVENTION

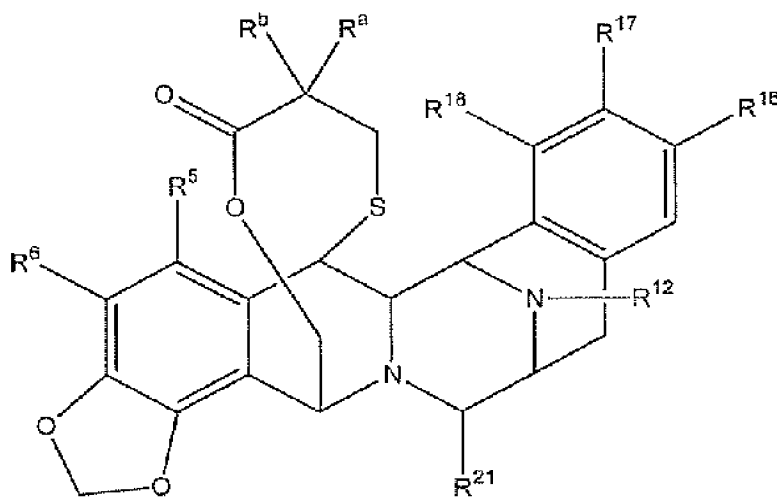
Monocyte/macrophages are recognized important components of innate and adaptive immunity. Circulating monocytes are versatile precursors with the ability to differentiate into the various forms of tissue macrophages. Macrophages stand guard against foreign invaders and are able to instantly defend the body against pathogens, as well as send signals for recruitment of other immunocompetent cells and present antigen to T lymphocytes. On the other hand, macrophages have also been implicated in the onset or progression of several diseases, mainly via their production of pro-inflammatory and proangiogenic mediators. Such conditions include, for instance, the pronounced inflammation present in several chronic diseases (e.g.: rheumatoid arthritis, atherosclerosis, lupus erythematosus) and tumours.

At the tumour site, Tumour-Associated Macrophages (TAM) represent a major component of infiltrating stromal cells. TAM have a complex ambiguous role within tumours, as suggested in the macrophage balance hypothesis. In fact, although macrophages stimulated with LPS and IFN gamma (also called M1 macrophages or classically activated macrophages) have the potential to kill tumour cells, several lines of evidence support the idea that macrophages within the tumour microenvironment are skewed towards alternatively activated macrophages, or M2 macrophages. Most frequently TAM are non-cytotoxic and produce several growth and angiogenic

factors. TAM produce also immunosuppressive molecules (e.g. IL-10, TGFb) and a variety of inflammatory mediators, including chemokines. Chemokines activate matrix metalloproteases which digest matrix proteins and promote tumour dissemination. Thus, the accumulation of TAM at the tumour site and the continuous expression of inflammatory molecules may actually favour tumour progression.

#### SUMMARY OF THE INVENTION

Ecteinasclidin compounds include natural and synthetic compounds. They possess a fused five ring system, and a 1, 4 bridge. We have found anti-inflammatory activity in the ecteinasclidin compounds. Such compounds have been widely described, and may have the following general formula (I):



wherein:

R<sup>5</sup> is OH, alkoxy or alkanoyloxy;

R<sup>6</sup> is hydrogen, alkyl, alkenyl, alkynyl or aryl;

R<sup>12</sup> is hydrogen, alkyl, alkenyl, alkynyl or aryl;

R<sup>16</sup> is hydrogen, alkyl, alkenyl, alkynyl or aryl;

R<sup>17</sup> is OH, alkoxy or alkanoyloxy;

R<sup>18</sup> is OH, alkoxy or alkanoyloxy;

R<sup>21</sup> is H, OH, CN or another nucleophilic group; and

R<sup>a</sup> is hydrogen and R<sup>b</sup> is optionally substituted amino, or

R<sup>a</sup> with R<sup>b</sup> form a carbonyl function =O, or

R<sup>a</sup>, R<sup>b</sup> and the carbon to which they are attached form a tetrahydroisoquinoline group.

Thus, the present invention provides a method of treating inflammation which comprises administration of an effective amount of an ecteinascidin having a general formula (I).

The invention also provides medicaments comprising an ecteinascidin having a general formula (I), together with a pharmaceutically acceptable carrier or diluent.

The invention further provides the use of an ecteinascidin having a general formula (I) in the preparation of a medicament for use in the treatment of inflammation.

#### DETAIL DESCRIPTION OF THE INVENTION

We have found that ecteinascidin compounds possess anti-inflammatory activity. Thus, the present invention relates to a new medical indication for compounds of general formula (I) as defined above.

In these compounds the substituents can be selected in accordance with the following guidance:

Alkyl and alkoxy groups preferably have from 1 to 12 carbon atoms. One more preferred class of alkyl and alkoxy groups has from 1 to about 6 carbon atoms, and most preferably 1, 2, 3 or 4 carbon atoms. Methyl, ethyl and propyl including isopropyl are particularly preferred alkyl groups in the compounds of the present invention. Methoxy, ethoxy and propoxy including isopropoxy are particularly preferred alkyl groups in the compounds of the present invention. Another more preferred class of alkyl and alkoxy groups has from 4 to about 12 carbon atoms, yet more preferably from 5 to about 8 carbon atoms, and most preferably 5, 6, 7 or 8 carbon atoms. As used herein, the term alkyl, unless otherwise modified, refers to both cyclic and noncyclic groups, although cyclic groups will comprise at least three carbon ring members.

Preferred alkenyl and alkynyl groups in the compounds of the present invention have one or more unsaturated linkages and from 2 to about 12 carbon atoms. One more preferred class of alkenyl or alkynyl groups has from 2 to about 6 carbon atoms, and most preferably 2, 3 or 4 carbon atoms. Another more preferred class of alkenyl or alkynyl groups has from 4 to about 12 carbon atoms, yet more preferably from 5 to about 8 carbon atoms, and most preferably 5, 6, 7 or 8 carbon atoms. The terms alkenyl and alkynyl as used herein refer to both cyclic and noncyclic groups.

Suitable aryl groups in the compounds of the present invention include single and multiple ring compounds, including multiple ring compounds that contain separate and/or fused aryl groups. Typical aryl groups contain from 1 to 3 separated or fused rings and from 6 to about 18 carbon ring atoms. Specially preferred aryl groups include substituted or unsubstituted phenyl, naphthyl, biphenyl, phenanthryl and anthracyl.

Suitable alkanoyloxy and alkanoyl groups have from 2 to about 20 carbon atoms, more preferably from 2 to about 8 carbon atoms, still more

preferably from 2 to about 6 carbon atoms, even more preferably 2 carbon atoms. Another preferred class of alkanoyloxy groups has from 12 to about 20 carbon, yet more preferably from 14 to about 18 carbon atoms, and most preferably 15, 16, 17 or 18 carbon atoms.

The groups above mentioned may be substituted at one or more available positions by one or more suitable groups such as OR', =O, SR', SOR', SO<sub>2</sub>R', NO<sub>2</sub>, NHR', N(R')<sub>2</sub>, =N-R', NHCOR', N(COR')<sub>2</sub>, NHSO<sub>2</sub>R', CN, halogen, C(=O)R', CO<sub>2</sub>R', OC(=O)R' wherein each of the R' groups is independently selected from the group consisting of H, OH, NO<sub>2</sub>, NH<sub>2</sub>, SH, CN, halogen, =O, C(=O)H, C(=O)CH<sub>3</sub>, CO<sub>2</sub>H, substituted or unsubstituted C<sub>1</sub>-C<sub>12</sub> alkyl, substituted or unsubstituted C<sub>2</sub>-C<sub>12</sub> alkenyl, substituted or unsubstituted C<sub>2</sub>-C<sub>12</sub> alkynyl and substituted or unsubstituted aryl. Suitable halogen substituents in the compounds of the present invention include F, Cl, Br and I.

Preferred compounds of the invention are those of general formula (I) wherein one or more of the following definitions will apply:

R<sup>5</sup> is an alkanoyloxy;

R<sup>6</sup> is methyl;

R<sup>12</sup> is methyl;

R<sup>16</sup> is methyl;

R<sup>17</sup> is methoxy;

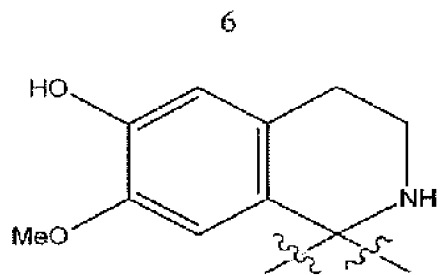
R<sup>18</sup> is OH;

R<sup>21</sup> is H, OH or CN; and

R<sup>a</sup> is hydrogen and R<sup>b</sup> is an amido group, or

R<sup>a</sup> with R<sup>b</sup> form =O, or

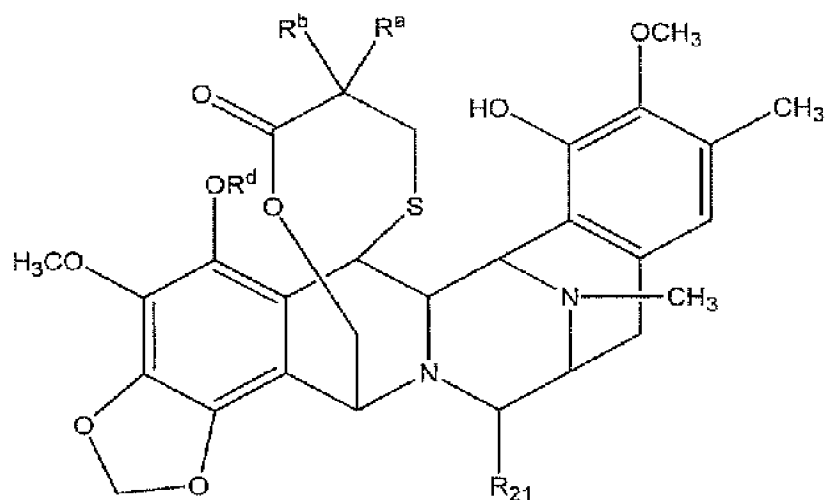
R<sup>a</sup>, R<sup>b</sup> and the carbon to which they are attached form a group of formula (II):



Examples of compounds for the present invention include natural ecteinascidins, such as ecteinascidin 743 and other 1,4 bridged fused ecteinascidin compounds disclosed for example in US 5,089,273, US 5,478,932, US 5,654,426, US 5,721,362, US 6,124,293, US 5,149,804, US 09/546,877, US 5,985,876 and WO 01/77115.

Ecteinascidin 743, also known as ET743 or ecteinascidin 743 is particularly preferred. ET743 is a natural product derived from the marine tunicate *Ecteinascidia turbinata*, with potent anti-tumor activity. It is a novel effective drug that is currently in clinical trials and has shown anti-cancer activity in some human solid tumors, including soft tissue sarcomas, breast and ovarian cancer.

Compounds of the following formula (III) are particularly preferred:

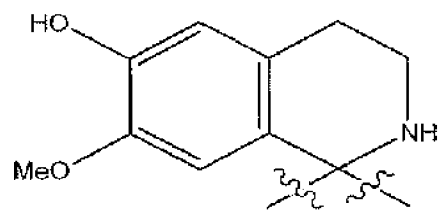


where

$R^a$  is hydrogen and  $R^b$  is amido of formula  $-NHR^f$  where  $R^f$  is alkanoyl, or

R<sup>a</sup> with R<sup>b</sup> form =O, or

R<sup>a</sup>, R<sup>b</sup> and the carbon to which they are attached form a group of formula (II):

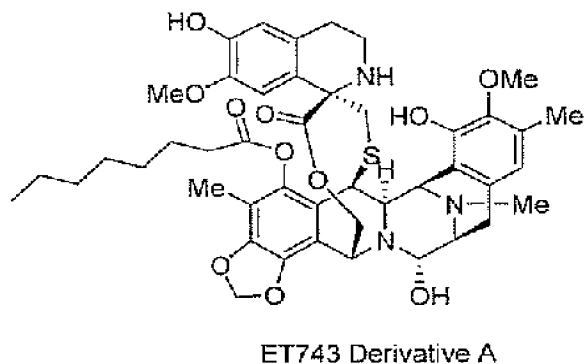
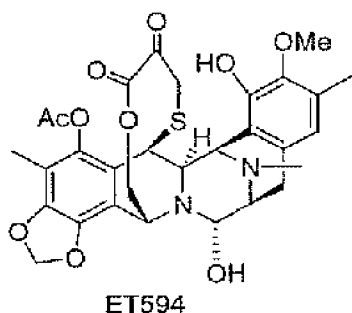
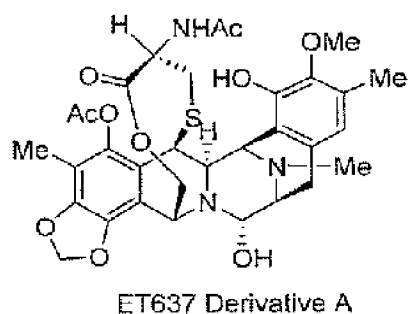
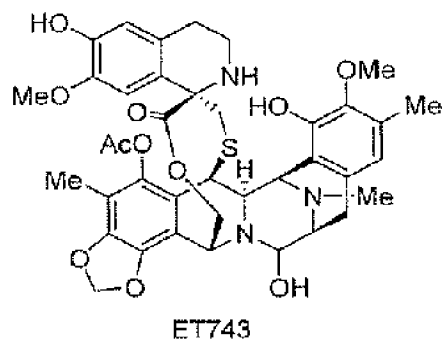


R<sup>d</sup> is alkanoyl; and

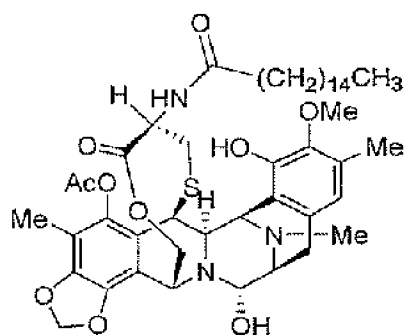
R<sup>21</sup> is H, OH or CN.

The alkanoyl groups can be acetyl or higher, for example up to C<sub>20</sub>.

Thus, preferred compounds of this invention include:

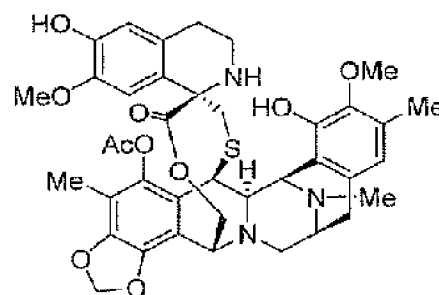






ET637 Derivative B

or



ET745

and related compounds with different acyl groups.

The medicaments provided by this invention are pharmaceutical compositions comprising the ecteinascidin compound and a pharmaceutically acceptable carrier. Medicaments can be of conventional form, and suitable dosing procedures can be devised.

As it has been indicated, the compounds of the invention are useful as anti-inflammatory agents. Thus, these compounds can be used in the treatment of diseases that deal with inflammation, particularly in the treatment of chronic inflammatory and autoimmune diseases (e.g. rheumatoid arthritis, Sjogren disease, Crohn disease) and for atherosclerosis.

#### DRAWINGS

Fig. 1. Panel A: Cell viability of blood monocytes, lymphocytes and thymocytes cultured with ecteinascidin 743.

Fig. 1. Panel B: Apoptosis of monocytes treated with ecteinascidin 743.

Fig. 2. Pre-treatment with M-CSF partially protects monocytes from the pro-apoptotic effect of ecteinascidin 743.

Fig. 3. Panel A: Kinetics of the cytotoxic effect of ecteinascidin 743 on monocytes.

Fig. 3. Panel B: Inhibition of macrophage differentiation.

Fig. 4. Panel A: Susceptibility to ET743 of monocytes and macrophages from the same donor.

Fig. 4. Panel B: Susceptibility to ET743 of macrophages classically activated by LPS and IFN $\gamma$  or by IL-4.

Fig. 4. Panel C: Susceptibility to ET743 of Tumour-Associated Macrophages (TAM).

Fig. 5. *In vivo* infusion of ecteinascidin 743 in tumour patients induces transient monocytopenia.

Fig. 6. Ecteinascidin 743 inhibits CCL2 (Panel A) and IL-6 (Panel B) production by monocytes and macrophages.

Fig. 7. Ecteinascidin 743 inhibits CCL2 (Panel A) and IL-6 (Panel B) production in TAM and in freshly isolated tumour cells.

Fig. 8. Panel A: Ecteinascidin 743 does not affect TNF production by monocytes, macrophages and TAM .

Fig. 8. Panel B: Real time-PCR of CCL2 and TNF transcripts in LPS-stimulated monocytes exposed to ecteinascidin 743.

Fig. 9. Panel A: Cytotoxicity of ecteinascidin 743 , Doxorubicin, Taxol and Cis-DDP on monocytes. The asterisc indicates the IC50 for each drug on in vitro cultured tumour cell lines.

Fig. 9. Panel B: CCL2 and TNF production by LPS-stimulated monocytes treated with the indicated doses of anti-tumour agents.

Fig. 10. CCL2 secretion by LPS-monocytes pre-treated with ecteinascidin 743 and other ecteinascidin compounds.

#### EXAMPLES OF THE INVENTION

In this study we demonstrate that, at concentrations within the pharmacological range, ecteinascidin 743 showed selective toxicity for the myeloid lineage and induced apoptosis of monocyte/macrophages. At non cytotoxic concentrations ecteinascidin 743 significantly inhibited in vitro macrophage differentiation and reduced the production of selected inflammatory cytokines. These findings may be relevant for therapeutic approaches aimed at targeting monocyte/macrophages in several human diseases.

In addition to ET743, ET637 Derivative A, ET637 Derivative B, ET594, ET743 Derivative A and ET745 were also tested. They have also been shown to reduce the production of selected inflammatory cytokines.

#### **Materials and Methods**

##### *Cell preparation:*

Purified populations of human blood monocytes were prepared as previously described by differential density centrifugation on Ficoll and Percoll gradients (see Allavena, P., Piemonti, L., Longoni, D., Bernasconi, S., Stoppacciaro, A., Ruco, L., and Mantovani, A. IL-10 prevents the differentiation of monocytes to dendritic cells but promotes their maturation

to macrophages. Eur J Immunol, 28: 359-369, 1998). Monocytes were usually >85% CD14+ cells. Purified T lymphocytes (>95% CD3+) were obtained on Percoll gradients as previously described (see Chieppa, M., Bianchi, G., Doni, A., Del Prete, A., Sironi, M., Laskarin, G., Monti, P., Piemonti, L., Biondi, A., Mantovani, A., Introna, M., and Allavena, P. Cross-linking of the mannose receptor on monocyte-derived dendritic cells activates an anti-inflammatory immunosuppressive program. J Immunol, 171: 4552-4560, 2003). Human thymocytes were isolated from resected thymus from pediatric patients undergoing surgery. Thymocytes were obtained by teasing and isolated on Percoll gradient.

Cells were cultured at 10<sup>6</sup> cells/ml in complete medium RPMI (Biochrom, Berlin, FRG)+ 10% FCS (Hyclone, Logan, UT). In vitro differentiated macrophages were obtained by culture of monocytes Monocyte-Colony Stimulating Factor (M-CSF) Peprotech (20 ng/ml), for 5 days. In some experiments, macrophages were treated with LPS (100 ng/ml) Sigma Aldrich, IFN gamma (500 IU/ml) or IL-4 (20 ng/ml) (Schering Plough) for 24 h.

Tumour-associated macrophages (TAM) and tumour cells were isolated from the ascitic fluid of patients with diagnosed ovarian adenocarcinoma, admitted to the Clinic of Obstetrics and Gynecology of the University of Milan-Bicocca, S Gerardo Hospital. Cells contained in the ascitic fluid were centrifuged and isolated by differential density gradients of Ficoll and Percoll, and plastic adherence as previously described (see Allavena, P., Peccatori, F., Maggioni, D., Erroi, A., Sironi, M., Colombo, N., Lissoni, A., Galazka, A., Meiers, W., Mangioni, C., *et al.* Intraperitoneal recombinant gamma-interferon in patients with recurrent ascitic ovarian carcinoma: modulation of cytotoxicity and cytokine production in tumour-associated effectors and of major histocompatibility antigen expression on tumour cells. Cancer Res, 50: 7318-7323, 1990). Purity of TAM and tumour cell preparations was

usually  $> 65 \pm 10\%$  as defined by morphology and phenotype analysis. Cells were treated with ecteinascidin 743 at the indicated concentrations and cultured for 1-5 days, as specified in figure legends. At the end of the incubation period cells were collected, washed and used for DNA analysis or functional assays.

*Determination of cell viability.*

Cell viability was analyzed by DNA content in Flow Cytometry

Cells exposed to treatments were fixed with ethanol 70%, washed in PBS and stained with propidium iodide (PI) solution containing 10 ug/ml PI in PBS and 25  $\mu$ l RNase 10,000 units, overnight in the dark. PI incorporation was evaluated on at least 20.000 cells/sample using a FACS Calibur instrument (Becton Dickinson, Sunnyvale, CA, USA), with a bandpass filter at 620 nm. Apoptosis was detected by staining with AnnexinV and PI. FACS analysis was performed using a bandpass filter 530 and 620 nm for green (AnnexinV) and red (PI) fluorescence respectively, in combination with a 570 nM dichroic mirror.

*Phenotype analysis.*

Expression of cell membrane markers was performed by immunofluoresce and analyzed by Flow Cytometry. Cells were incubated with anti-CD14, anti-CD16, anti-CD68 , anti-CD206 (mannose receptor) and then with FITC-goat anti-mouse Ig as described. At least 10.000 cells were analyzed.

*Cytokine production.*

Supernatants of untreated cells or cells treated with ecteinascidin 743 or other anti-neoplastic agents were collected after 24 h culture and frozen. Monocytes, macrophages and TAM were stimulated with 100 ng/ml LPS to induce maximal cytokine production. Determination of cytokines CCL2, TNF and IL-6 was measured by specific ELISA following the manufacturer's instructions.

#### *Tumour patients.*

Patients with sarcoma or ovarian cancer undergoing Phase II trial with ecteinascidin 743, were admitted to the European Oncology institute, Milano, Italy. Patients received ecteinascidin 743 (1300 mg/m<sup>2</sup>) in a 3-h infusion. Blood samples (40 ml) were collected immediately before the treatment and at the end of the infusion (+3 h). Blood samples were immediately processed and Percoll purified monocytes (usually 10<sup>6</sup> cells) were cultured with M-CSF (20 ng/ml) for 5 days. Differentiated cells were harvested, counted and analyzed for phenotype expression, as described above. Results are presented as absolute numbers of marker-positive cells/10.000 cells. Significant inhibition of macrophage differentiation was considered a 50% reduction of marker+ cells, relative to cells collected before therapy, from the same patient.

#### EXAMPLE 1

##### *Ecteinascidin 743 shows selective cytotoxic effect on mononuclear phagocytes*

We first studied the effect of ecteinascidin 743 treatment on the viability of human leukocyte subsets *in vitro*. Purified preparations of blood monocytes, lymphocytes and thymocytes were cultured with different concentrations of ecteinascidin 743 for 48 h. Cell viability was assessed by

DNA analysis and propidium iodide (PI) staining in Flow cytometry. Purified preparations of blood monocytes were highly susceptible to the cytotoxic effect of the drug. There was a dose-dependent mortality with a lethal dose 50% (IC<sub>50</sub>) of 2.5-5 nM after 48 h of culture (Fig.1A). Purified T lymphocytes were much less susceptible and at 5 nM were all alive. IC<sub>50</sub> for lymphocytes was 20 nM. Even more resistant were freshly isolated thymocytes (IC<sub>50</sub> >40 nM, Fig.1A).

Virtually all dying monocytes exposed to ecteinascidin 743 stained positive for Annexin V, indicating that the drug induces apoptosis (Fig.1B). Monocyte mortality was confirmed also by DNA analysis in Flow Cytometry (Fig.2). In the presence of M-CSF, a growth and differentiation factor for monocytes, a partial protection from the toxic effect of ecteinascidin 743 was observed. M-CSF shifted monocyte death from 55% to 30% at 5 nM ecteinascidin 743, after 48 h incubation, and from 65% to 35% at 10 nM, after 24 h treatment (Fig.2). M-CSF was effective only if added simultaneously or before ecteinascidin 743, but was no longer effective when given 4 h after the drug.

A kinetics analysis of the cytotoxic effect of ecteinascidin 743 was performed in the presence of M-CSF. Cells were treated with M-CSF (20 ng/ml) and different concentrations of ecteinascidin 743. Samples were collected at the indicated times and tested for DNA analysis. At higher concentrations, significant toxicity was observed already after 24 h incubation and increased over time (Fig.3A). Lower concentrations (2.5 nM) induced 40-50% mortality after 5 days.

We next studied the effect of ecteinascidin 743 on already differentiated macrophages obtained from monocytes cultured in vitro for 5 days with M-CSF. The addition of ecteinascidin 743 in the last 48 h resulted in significant mortality, but to a lower extent compared to freshly isolated

monocytes. Fig.4A shows a representative experiment comparing the susceptibility of monocytes and macrophages from the same donor. Monocytes were differentiated to macrophages by culture with M-CSF (20 ng/ml). At day 3, ecteinascidin 743 was added to cultures and incubated for 48 h. Results show the comparison of monocytes and macrophages obtained from the same donor. Viability was assessed by PI staining and analyzed by Flow Cytometry. Similar results were obtained in other 4 experiments. In a series of 4 different experiments, IC<sub>50</sub> for in vitro differentiated was 10 nM.

We then tested the susceptibility to ecteinascidin 743 of macrophages classically activated by LPS and IFN gamma (or M1 macrophages) and alternatively activated by IL-4 (or M2 macrophages). In vitro differentiated macrophages were stimulated with LPS (100 ng/ml)+ IFNgamma (500 UI/ml), IL-4 (20 ng/ml), in the presence or absence of ecteinascidin 743 for 48h. Viability was assessed by PI staining and analyzed by Flow Cytometry. Both LPS-stimulated and IL-4-stimulated macrophages were susceptible to drug treatment similarly as non-stimulated macrophages (Fig.4B).

We also tested Tumour-Associated Macrophages (TAM) isolated from the ascites of non-treated ovarian adenocarcinoma patients. Enriched preparations of TAM isolated from three different patients with ovarian cancer were treated in vitro with ecteinascidin 743 for 48h. Viability was assessed by PI staining and analyzed by Flow Cytometry. TAM were significantly killed *in vitro* by ecteinascidin 743 with 40-70% mortality at 10 nM. Results from three different patients are shown in Fig.4C.

Overall these experiments demonstrate that human mononuclear phagocytes are highly susceptible to the cytotoxic effect of ecteinascidin 743 at concentrations within the therapeutic range. It should be noted that even in the presence of M-CSF, monocytes never underwent cell cycle



progression, as checked by DNA analysis with flow cytometry. The toxic effect of ecteinascidin 743 on monocytes is therefore independent from cell cycle and provides the unique opportunity to study the biological effects of this drug on non-replicating cells.

## EXAMPLE 2

### *Non-cytotoxic concentrations of ecteinascidin 743 inhibit in vitro and in vivo macrophage differentiation*

In order to study the effect of ecteinascidin 743 on macrophage differentiation, non cytotoxic doses of the drug were used. Monocytes were cultured with M-CSF (20 ng/ml) and with sub-cytotoxic concentrations of ecteinascidin 743 for 5 days. Phenotype analysis was performed by indirect immunofluorescence and analyzed in Flow Cytometry by gating on large cells. Usually, an average of  $65 \pm 15\%$  (mean  $\pm$ SD of  $> 10$  experiments) of input monocytes differentiate into large cells expressing typical macrophage markers, including CD16, CD68 and CD206 (mannose receptor). After 5 days of culture monocyte viability, evaluated by propidium iodide staining in flow cytometry, was 92% and 70% of untreated cells at 0.5 and 1 nM ecteinascidin 743, respectively. The process of macrophage differentiation was partially inhibited as the de novo expression of CD68, CD16 and CD206 was reduced at 1 nM ecteinascidin 743 (Fig.3B).

To validate the above in vitro findings we tested whether the in vivo administration of ecteinascidin 743 in tumour patients could have measurable effects on monocyte viability and capacity to macrophage differentiation in vitro. A phase II trial with ecteinascidin 743 is currently underway in advanced ovarian adenocarcinoma patients who had failed two different cycles of conventional cis-platin and taxol-based chemotherapy.

Tumour patients selected for this study were treated with 1300 ug/ml/m<sup>2</sup> of ecteinascidin 743. Blood samples from patients were drawn just before drug administration and at the end of a 3-hour infusion. Purified monocytes were immediately isolated and cultured with M-CSF (20 ng/ml) for 5 days to induce macrophage differentiation and then analyzed for phenotype expression. Of 12 evaluable patients, monocytes from 6 subjects showed decreased macrophage differentiation after ecteinascidin 743 treatment. Table 1 shows the phenotype analysis of in vitro differentiated macrophages from patients whose cells after therapy showed at least 50% inhibition of CD206, CD16 and CD68 expression, compared to cells collected before therapy. The data shown are the absolute numbers of marker positive cells for a total of 10,000 input cells. Monocytes collected from the other six patients did not show any significant decrease in their differentiation capacity.

TABLE 1. Effect of in vivo treatment with ecteinascidin 743 on the in vitro differentiation of macrophages in tumour patients.

Patients	Absolute numbers of marker positive macrophages/10,000 cells		
	No exposure	Exposure to ecteinascidin 743	% inhibition*
UPN 1			
CD206	4350	550	88
CD16	3110	1248	60
CD68	2703	1473	45
UPN 2			
CD206	2810	595	79
CD16	2705	1105	60
CD68	3500	1060	70

UPN 3			
CD206	3590	474	87
CD68	3260	632	80
UPN 4			
CD16	5130	3050	41
CD68	5550	2460	55
UPN 5			
CD16	1575	594	63
CD68	1620	815	50
UPN 6			
CD16	2750	480	83
CD68	2320	505	79

\* % inhibition of macrophage differentiation referred to cells before infusion.

We also investigated whether the in vivo treatment with ecteinascidin 743 caused a measurable monocytopenia in cancer patients. Monocyte values were obtained from blood formula during routine clinical analysis. Of 9 patients whose morphological analysis of monocytes was recorded and available, 7 patients showed a decrease ( 25% inhibition compared to values before infusion, in at least one cycle) in the number of monocytes, evaluated both as % of monocytes over total leukocytes, and as absolute number of monocytes/ul of blood. Results from three representative patients are shown in Fig. 5. In spite of a constant level or a transient increase in the total number of leukocytes , in the first few days following drug infusion, monocytes never increased and actually were frequently decreased.

## EXAMPLE 3

*Ecteinascidin 743 inhibits the production of inflammatory cytokines/chemokines*

Monocytes/macrophages are potent producer of soluble factors which orchestrate the inflammatory/immune response. We therefore tested the effect of ecteinascidin 743 treatment on the secretory function of these cells. The chemokine CCL2 is a major chemoattractant for mononuclear phagocytes and is produced by immune as well as several tumour cells. Tumour-derived CCL2 attracts circulating monocytes at the tumour site and the TAM content of a tumour correlates with levels of CCL2, as demonstrated in several tumours.

Monocytes and in vitro differentiated macrophages were stimulated with LPS (100 ng/ml). After 1 h LPS stimulation they were treated with ecteinascidin 743. After 16 h incubation, cell supernatants were harvested and tested in ELISA. Under these treatment conditions cell viability was usually >85% for concentrations up to 5 nM. Treatment with ecteinascidin 743 dose-dependently reduced the production of CCL2 by LPS-stimulated monocytes and in vitro-derived macrophages (Fig.6A). Mean inhibition at 5 nM, for monocytes, was 65% (range 50-80%, n =5) and was 50% (range 25-75%, n=5) for in vitro differentiated macrophages. Results are mean +/- SE of 3-5 experiments

Next, TAM associated to ovarian carcinomas were tested. Freshly isolated ovarian tumor cells and TAM were incubated with ecteinascidin 743 for 16 h. TAM were stimulated with LPS (100 ng/ml). Cell supernatants were harvested and tested in ELISA. Results are mean +/- SE of 4 experiments for TAM and from 1 experiment for tumor cells. The LPS-

stimulated production of CCL2 was reduced by 50% (range 40-60%, n=4) (Fig.7A), while their constitutive production by 43% (range 30-50%, n=4).

We also tested two other cytokines, IL-6 and TNF, produced by macrophages and tumour cells, which have inflammatory properties and also act as growth factors for some tumours. IL-6 production was always reduced after ecteinascidin 743 treatment, with an overall inhibition at 5 nM of 54% (range 51-57%, n=2) and 69% (range 66-72%, n=2), in monocytes and macrophages, respectively (Fig.6B). IL-6 release in TAM was somehow more resistant to treatment : at 5 nM mean inhibition was 35% (range 25-53%, n=4); at 10 nM was 47% (range 33-63%, n=4), (Fig.7B).

Of interest, ecteinascidin 743 reduced also the constitutive production of CCL2 and IL-6 by freshly isolated tumour cells. A representative experiment is shown in Fig 7.

In contrast, and quite surprisingly, when monocytes, in vitro differentiated macrophages and TAM were stimulated with LPS (100 ng/ml), treated with ecteinascidin 743 preceeded of 1 h LPS stimulation, and after 16 h incubation, cell supernatants were harvested and tested in ELISA, it was observed that the production of TNF by monocytes/macrophages, as well as by TAM was never inhibited, even up to 10 nM for TAM (Fig.8A), suggesting that ecteinascidin 743 interferes only with selected genes. These results also indicate that, under these conditions cells were not damaged by the treatment. To verify whether the inhibitory effect of ecteinascidin 743 on cytokine production was at the transcriptional level, we analyzed mRNA of CCL2 and TNF from LPS-stimulated macrophages by real time-PCR of CCL2 and TNF transcripts in LPS-stimulated monocytes exposed to ecteinascidin 743. As shown in Fig. 8B, after ecteinascidin 743 treatment a consistent reduction of CCL2 transcripts was observed, while TNF mRNA was unaffected, in line with the results obtained in Elisa.

Overall these results indicate that ecteinascidin 743 at pharmacological concentrations reduces the production of two important inflammatory cytokines in mononuclear phagocytes and tumour cells.

#### EXAMPLE 4

*Other ecteinascidin compounds also inhibit the production of inflammatory cytokines/chemokines*

We also tested five other ecteinascidin compounds (Table 2) for their capacity to inhibit the production of CCL2 by human monocytes in vitro. Of the five compounds tested, only ET637 Derivative A showed marked and consistent ability to downmodulate inflammatory cytokine production by monocytes, at concentrations of 2.5 and 5 nM. These concentrations did not affect monocyte viability after 48 h of exposure. The extent of inhibition of ET637 Derivative A was even more pronounced compared to ET743. In Table 2 is shown that the production of CCL2, induced by exposure of monocytes to tumor cell supernatants, is inhibited up to 80% and 97% at 2.5 and 5 nM, respectively, in two different donors. In the same experiment ET743 inhibited between 30% and 70%. The other compounds also showed an inhibitory activity, but at a lower level than the other two above mentioned compounds.

Table 2. Inhibitory effect of ET743 and other ecteinascidin compounds on the production of the inflammatory chemotactic cytokine CCL2

		Donor A % inhibition	Donor B % inhibition
ET743	2.5 nM	30	60

	5 nM	70	70
ET637	2.5 nM	80	80
Derivative A	5 nM	97	87
ET594	5 nM	25	-
	10 nM	25	-
ET743	2.5 nM	30	-
Derivative A	5 nM	35	30
ET745	2.5 nM	-	-
	5 nM	23	-
ET637	2.5 nM	-	-
Derivative B	5 nM	25	-

Similar results were obtained when monocytes were stimulated with LPS (100 ng/ml) and treated with ET743 and the other ecteinascidin compounds, although the overall inhibition was less marked compared with the previous experiment where the tumor supernatant was used as CCL2-inducing stimulus.

In Fig. 10 it is confirmed that ET637 Derivative A gives a significant inhibition of CCL2 production.

#### EXAMPLE 5

*Comparison of ecteinascidin 743 with antineoplastic agents currently used in ovarian cancer*

As ecteinascidin 743 is being actively studying for the treatment of ovarian adenocarcinoma, it was of interest to compare these anti-inflammatory effects of ecteinascidin 743 with other compounds

conventionally used in this disease, namely Doxorubicin, Cisplatin and Taxol. Monocytes were incubated for 48 h with the indicated concentrations of ecteinascidin 743, Doxorubicin, Taxol and Cisplatin. Viability was assessed by PI staining and analyzed by Flow Cytometry. Fig.9A shows that at active concentrations on tumour cells ( $>0,5 \mu\text{M}$ ) Doxorubicin was highly cytotoxic on monocytes after 48 h treatment, while Cisplatin and Taxol were not. Significant toxicity with Cisplatin was observed only at very high concentrations ( $40 \mu\text{M}$ ), while Taxol was ineffective even at  $300 \text{ nM}$ .

CCL2 and TNF production by LPS-stimulated monocytes treated with the indicated doses of the anti-tumor agents was also tested. Cell supernatants were harvested after 24 h-incubation and tested in ELISA. As shown in Fig.9B, Taxol and Doxorubicin were ineffective, but DDP (Cisplatin) ( $10 \mu\text{M}$ ) reduced CCL2 production. None of these compounds interfered with the production of TNF. These results indicate that monocyte cytotoxicity and inhibition of CCL2 are not generalized properties of anti-tumour agents conventionally used in ovarian cancer treatment.

## DISCUSSION

In this study we have evaluated the cytotoxic effect of ecteinascidin 743 on mononuclear phagocytes. Blood circulating monocytes were highly susceptible to the drug and underwent apoptosis at concentrations of  $5 \text{ nM}/48 \text{ h}$ . In vitro differentiated macrophages and Tumour-Associated Macrophages (TAM) were also susceptible at  $5\text{-}10 \text{ nM}$ . These values are within the range of effective therapeutic concentrations. At low concentrations of ecteinascidin 743, monocytes were inhibited in their differentiation to macrophages. We have confirmed these results by studying monocytes from tumour-bearing patients undergoing ecteinascidin



743 therapy. In 6 of 12 patients tested, monocytes collected after 3 h infusion (1300 mg/m<sup>2</sup>) showed >50% inhibition of *in vitro* macrophage differentiation compared to monocytes collected just before therapy. Moreover, a significant monocitopenia has been observed in the first few days following drug infusion in the majority of the patients. These results indicate that a brief *in vivo* exposure to ecteinascidin 743 is sufficient to provide a cytotoxic effect on monocytes.

A major finding of our work is the inhibitory activity of ecteinascidin 743 on the production of inflammatory cytokines. Among various inflammatory cytokines produced by monocyte/macrophages we have tested IL-6, TNF and the chemokine CCL2. CCL2 is a chemokine attracting monocytes and other leukocyte subsets, and is produced both by monocyte/macrophages and several tumour cells. It has been described that ovarian adenocarcinoma cells produce huge amounts of CCL2 and that their levels correlate with the macrophage content of tumours. CCL2 is therefore one of the most important factors regulating monocyte/macrophages recruitment at the tumour site. Ecteinascidin 743 strongly inhibited CCL2 release by LPS-activated monocytes, macrophages and TAM. Ecteinascidin 743 also strongly inhibited the constitutive production of CCL2 by freshly isolated ovarian tumour cells. Thus, lower levels of CCL2 by TAM and tumour cells are likely to reduce the number of macrophages recruited at the tumour site. In the above described *in vitro* experiments ecteinascidin 743 was present throughout the 16-h culture period. We also checked whether a shorter *in vitro* exposure to ecteinascidin 743 was sufficient to affect cytokine production. Monocytes exposed to ecteinascidin 743 were washed after 1 hour culture and replaced in fresh medium. Under these conditions, inhibition of CCL2 production was still significant, though slightly lower compared to cells receiving 16 h-treatment (57% and 69% inhibition, respectively).

IL-6 is a pro-inflammatory cytokine with important effects on the immune/hematopoietic system and is a co-factor for the production of CCL2. In addition, several studies have pointed out that IL-6 may act as a growth factor for some tumour cells, including ovarian cancer. As for CCL2, the LPS-induced IL-6 was dramatically decreased in monocytes /macrophages by ecteinascidin 743. The constitutive IL-6 production of freshly isolated ascitic tumour cells was also reduced .

A novel, recently described effect of IL-6 is its ability to rescue T lymphocytes from the regulatory T cells (Treg)-mediated suppression. Treg are a small, albeit very important subset of T lymphocytes which control T cell auto-reactivity and maintain homeostasis. A role for Treg in auto-immune disease is well recognized. Auto-reactive T lymphocytes suppressed by Treg can be rescued by IL-6, thus perpetuating the auto-immune reaction. Therefore, the ecteinascidin 743 -mediated reduction of IL-6 could be a favourable therapeutic effect. Ecteinascidin 743 has never been considered for the treatment of chronic inflammatory disorders. The results of this study point out that both for its cytotoxic effect on precursors of antigen presenting cells (i.e. monocytes) and for its ability to decrease IL-6, ecteinascidin 743 is an interesting candidate in anti-inflammatory therapy.

Unlike CCL2 and IL-6, ecteinascidin 743 had no significant effect on the production of TNF, another important inflammatory mediator, produced by LPS-stimulated monocyte/macrophages.

We have demonstrated that other ecteinascidin compounds as well as ET743 are able to inhibit the production of CCL2 by human monocytes. From the compounds tested, ET637 Derivative A has showed marked and consistent ability to downmodulate CCL2 production. The extent of inhibition of ET637 Derivative A was even more pronounced compared to

ET743. The other compounds also showed an inhibitory activity, but at lower levels.

In conclusion, the finding that ecteinascidin 743 and the other ecteinascidin compounds affect viability and functions of monocyte/macrophages discloses novel effects of these compounds and a new therapeutic indication.

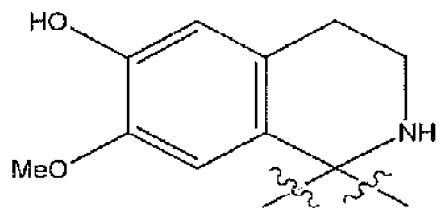


2. The method according to claim 1, wherein the inflammation is caused by a disease selected from the group consisting of chronic inflammatory diseases, autoimmune diseases and atherosclerosis.
3. The method according to claim 1, wherein in the ecteinascidin compound of formula (I), the group  $R^5$  is an alkanoyloxy.
4. The method according to claim 1, wherein in the ecteinascidin compound of formula (I), the group  $R^6$  is methyl.
5. The method according to claim 1, wherein in the ecteinascidin compound of formula (I), the group  $R^{12}$  is methyl.
6. The method according to claim 1, wherein in the ecteinascidin compound of formula (I), the group  $R^{16}$  is methyl.
7. The method according to claim 1, wherein in the ecteinascidin compound of formula (I), the group  $R^{17}$  is methoxy.
8. The method according to claim 1, wherein in the ecteinascidin compound of formula (I), the group  $R^{18}$  is OH.
9. The method according to claim 1, wherein in the ecteinascidin compound of formula (I), the group  $R^{21}$  is H, OH or CN; and

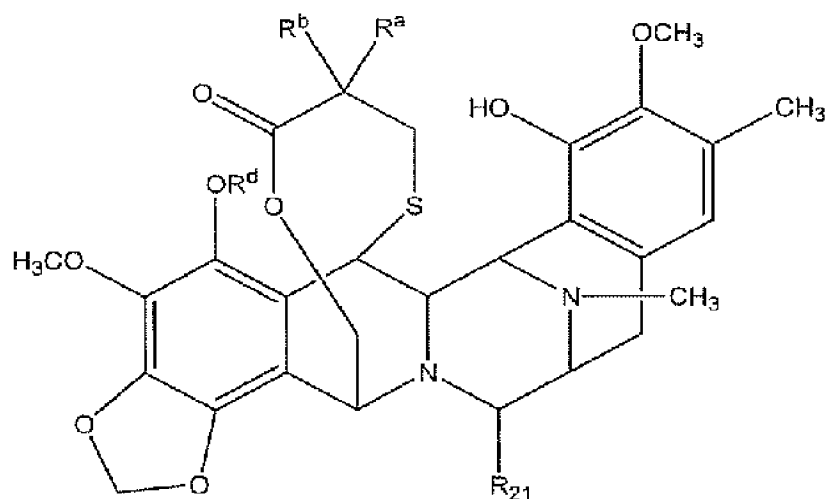
$R^a$  is hydrogen and  $R^b$  is an amido group, or

$R^a$  with  $R^b$  form  $=O$ , or

$R^a$ ,  $R^b$  and the carbon to which they are attached form a group of formula (II):



10. The method of claim 1, wherein the ecteinascidin comopund is of formula (III):



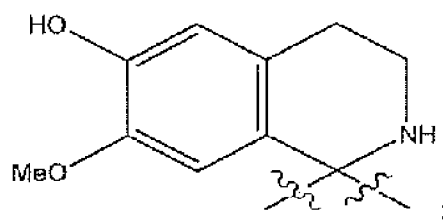
where

$R^a$  is hydrogen and  $R^b$  is amido of formula  $-NHR^f$  where  $R^f$  is alkanoyl, or

$R^a$  with  $R^b$  form  $=O$ , or

$R^a$ ,  $R^b$  and the carbon to which they are attached form a group of formula (II):

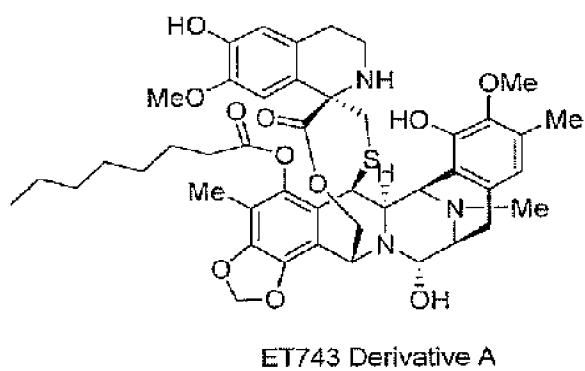
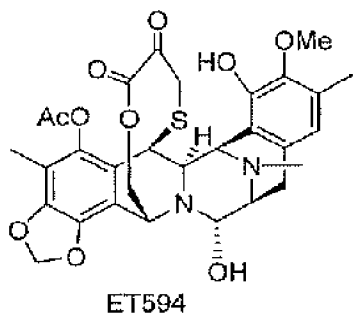
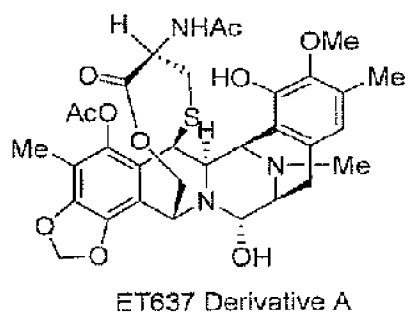
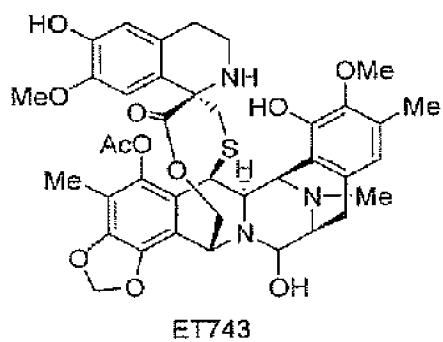
30



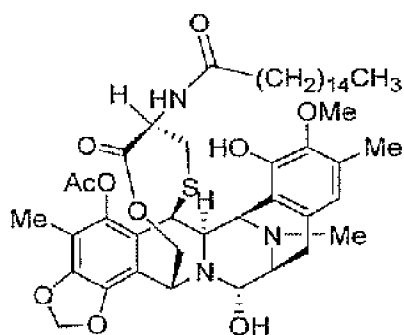
$R^d$  is alkanoyl; and

$R^{21}$  is H, OH or CN.

11. The method of claim 10, wherein the ecteinascidin compound is selected from the group consisting of:

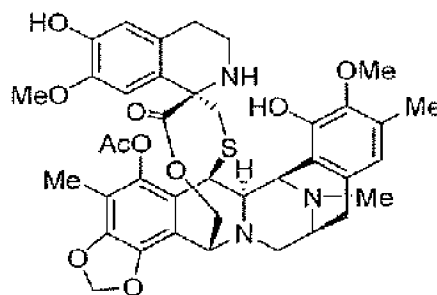


31



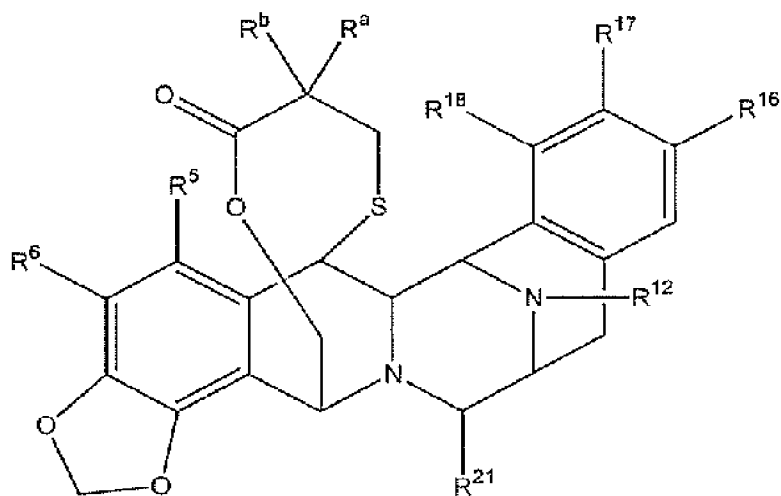
ET637 Derivative B

or



ET745

12. The use of an ecteinascidin compound of general formula (I):



wherein:

$R^5$  is OH, alkoxy or alkanoyloxy;

$R^6$  is hydrogen, alkyl, alkenyl, alkynyl or aryl;

$R^{12}$  is hydrogen, alkyl, alkenyl, alkynyl or aryl;

$R^{16}$  is hydrogen, alkyl, alkenyl, alkynyl or aryl;

$R^{17}$  is OH, alkoxy or alkanoyloxy;

$R^{18}$  is OH, alkoxy or alkanoyloxy;

$R^{21}$  is H, OH, CN or another nucleophilic group; and

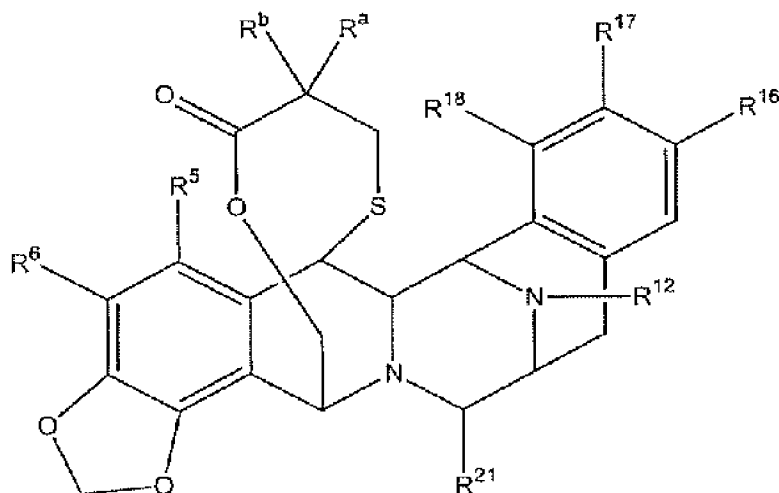
$R^a$  is hydrogen and  $R^b$  is optionally substituted amino, or

$R^a$  with  $R^b$  form a carbonyl function  $=O$ , or



Ra, Rb and the carbon to which they are attached form a tetrahydroisoquinoline group in the preparation of a medicament for use in a method according to any preceding claim.

13. A medicament for treatment of inflammation comprising an ecteinascidin compound of general formula (I):



wherein:

R<sup>5</sup> is OH, alkoxy or alkanoyloxy;

R<sup>6</sup> is hydrogen, alkyl, alkenyl, alkynyl or aryl;

R<sup>12</sup> is hydrogen, alkyl, alkenyl, alkynyl or aryl;

R<sup>16</sup> is hydrogen, alkyl, alkenyl, alkynyl or aryl;

R<sup>17</sup> is OH, alkoxy or alkanoyloxy;

R<sup>18</sup> is OH, alkoxy or alkanoyloxy;

R<sup>21</sup> is H, OH, CN or another nucleophilic group; and

R<sup>a</sup> is hydrogen and R<sup>b</sup> is optionally substituted amino, or

R<sup>a</sup> with R<sup>b</sup> form a carbonyl function =O, or

Ra, Rb and the carbon to which they are attached form a tetrahydroisoquinoline group, and a pharmaceutically acceptable carrier.

Figure 1

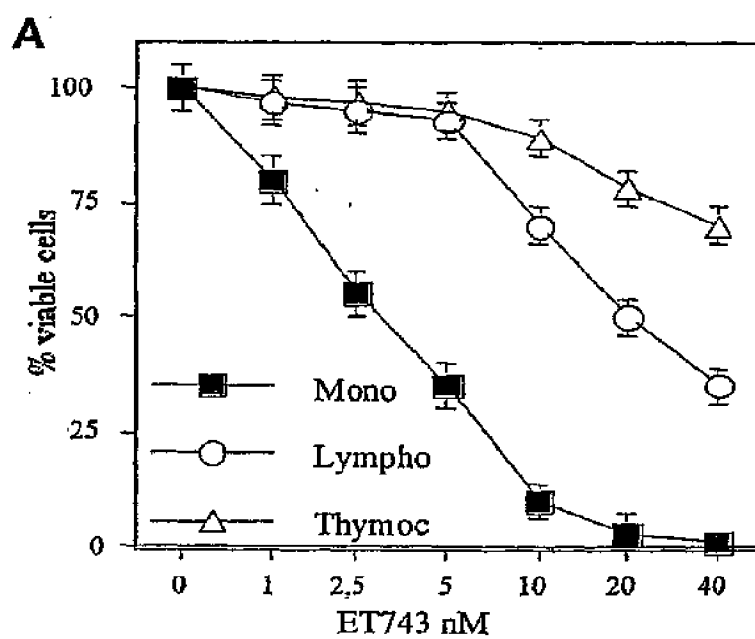


Figure 1

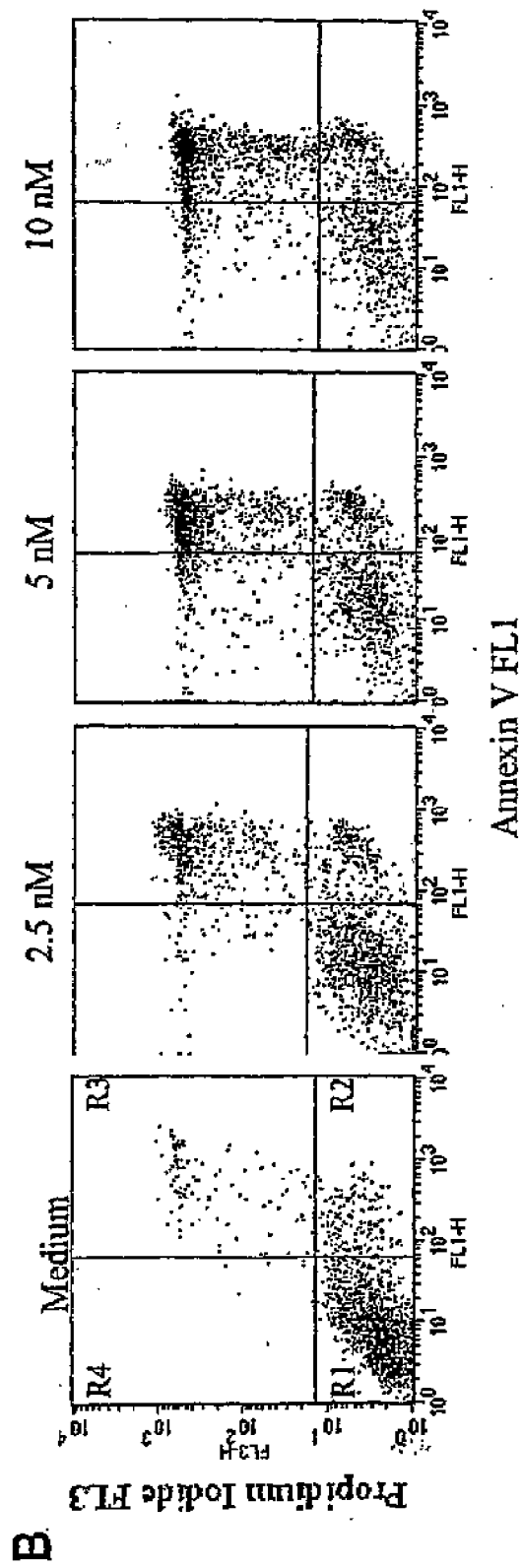


Figure 2

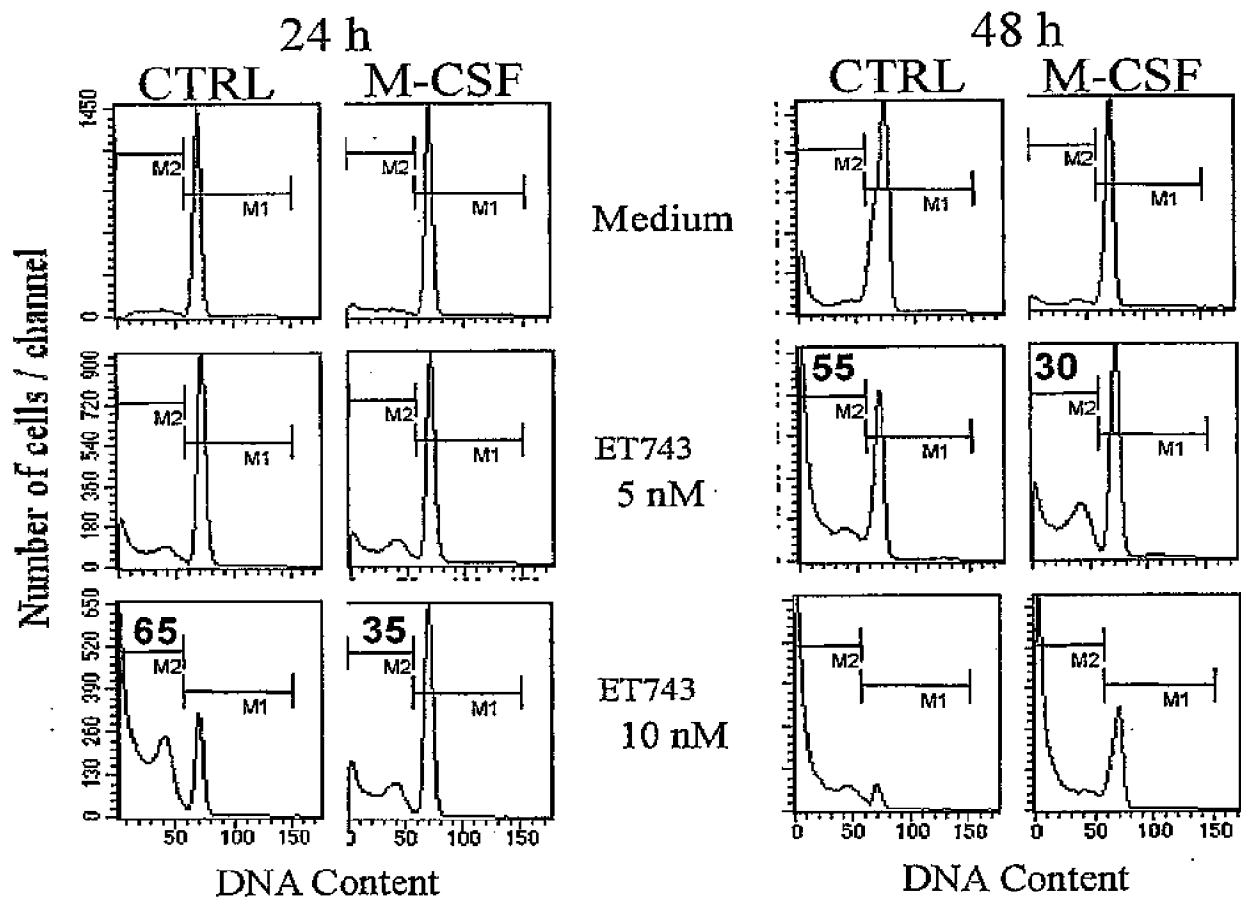


Figure 3

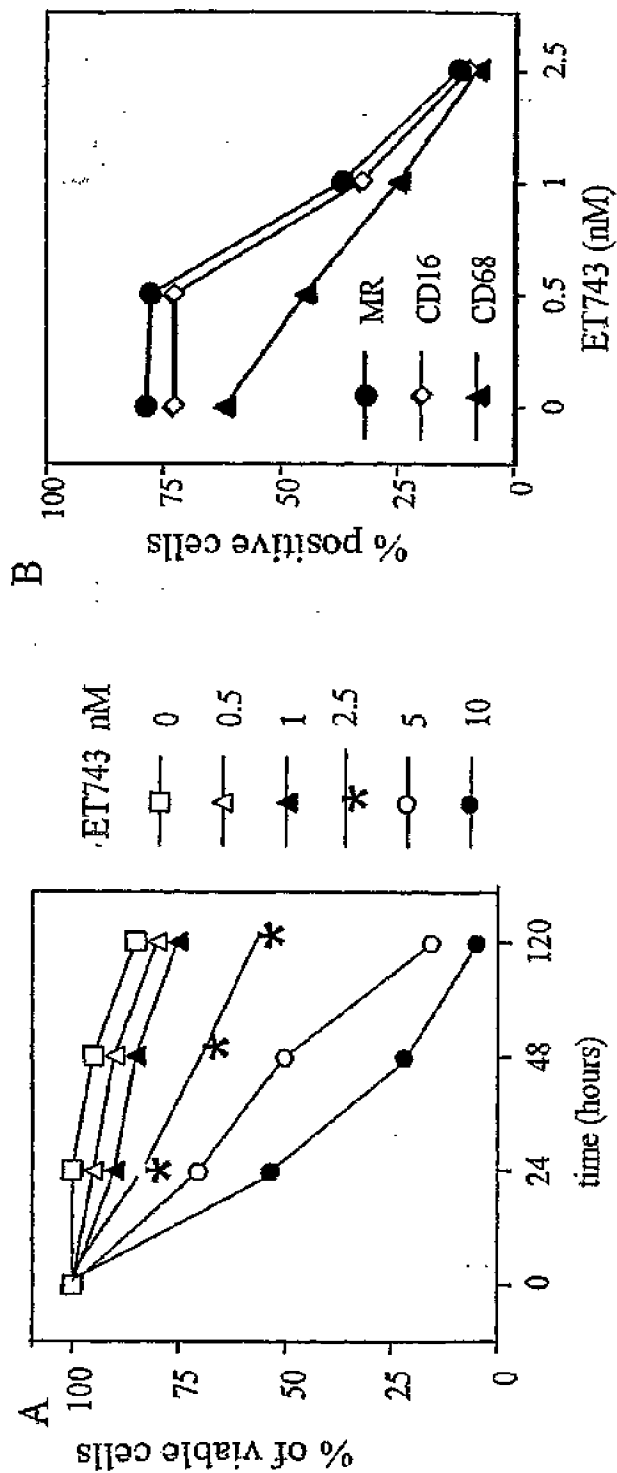


Figure 4

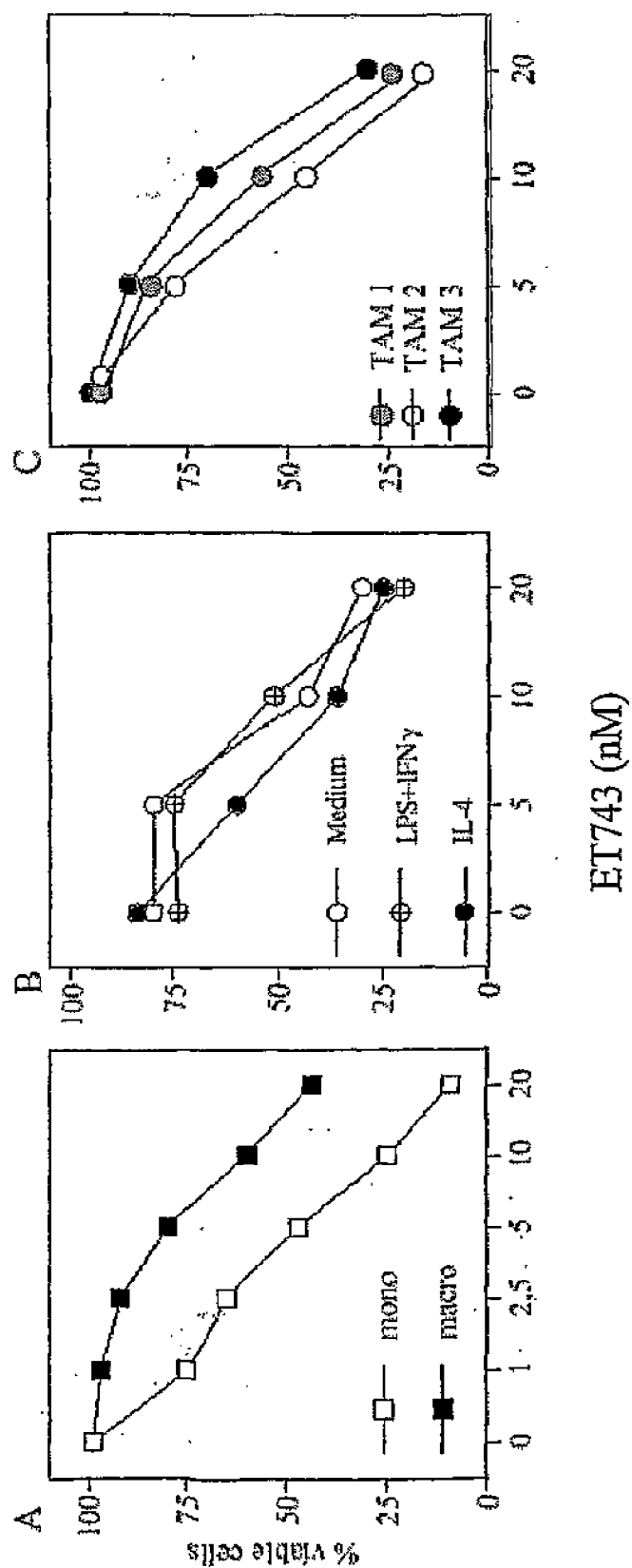


Figure 5

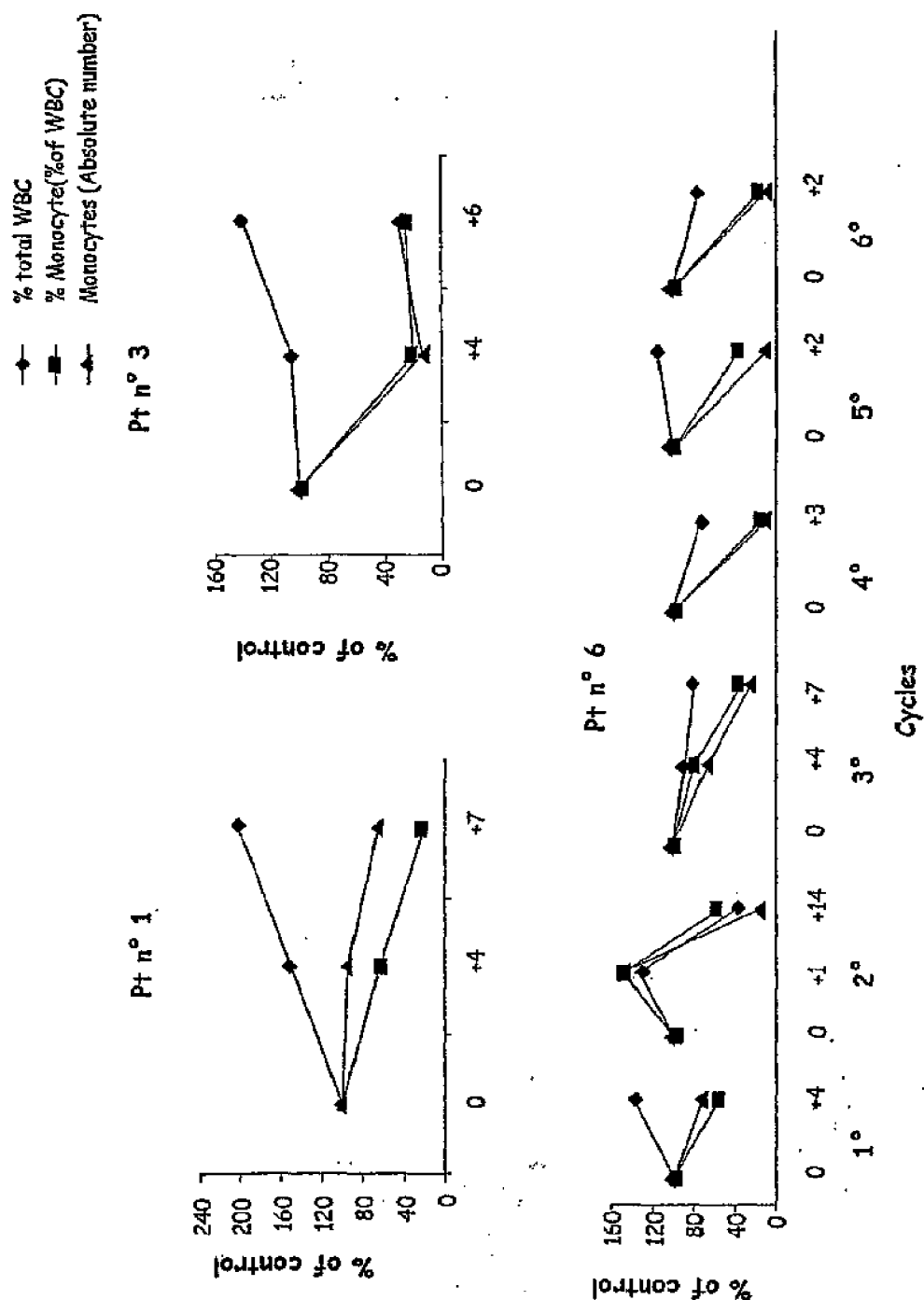


Figure 6

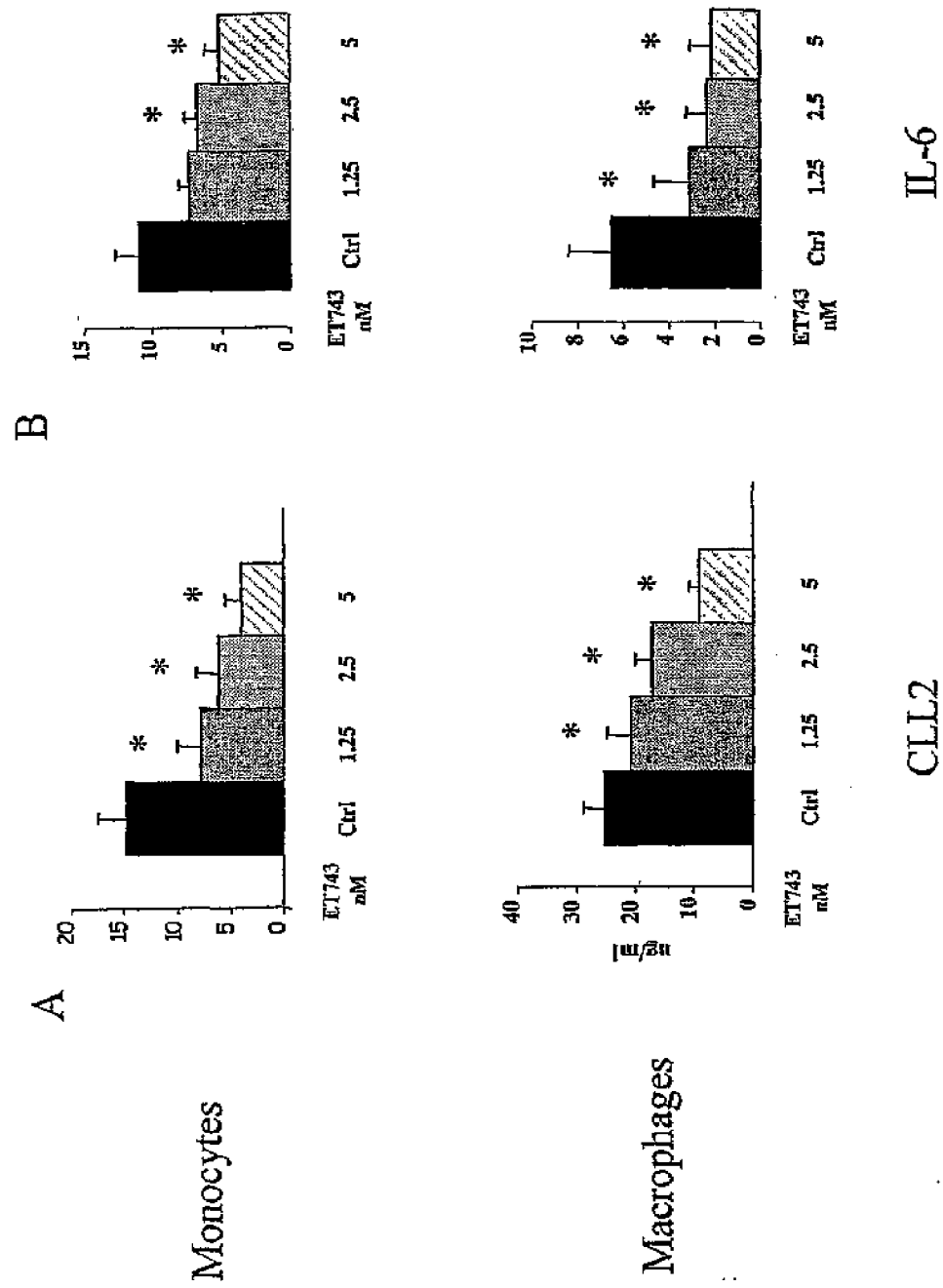




Figure 7

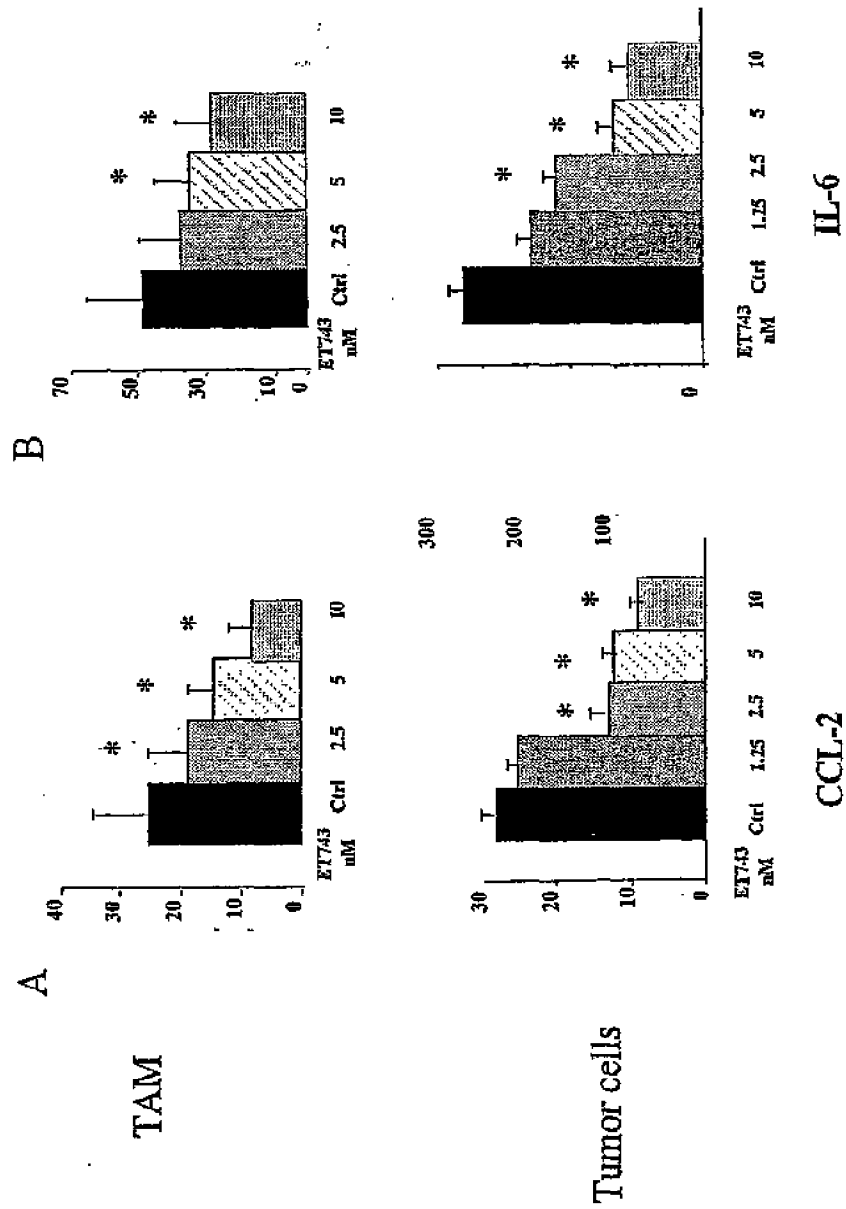
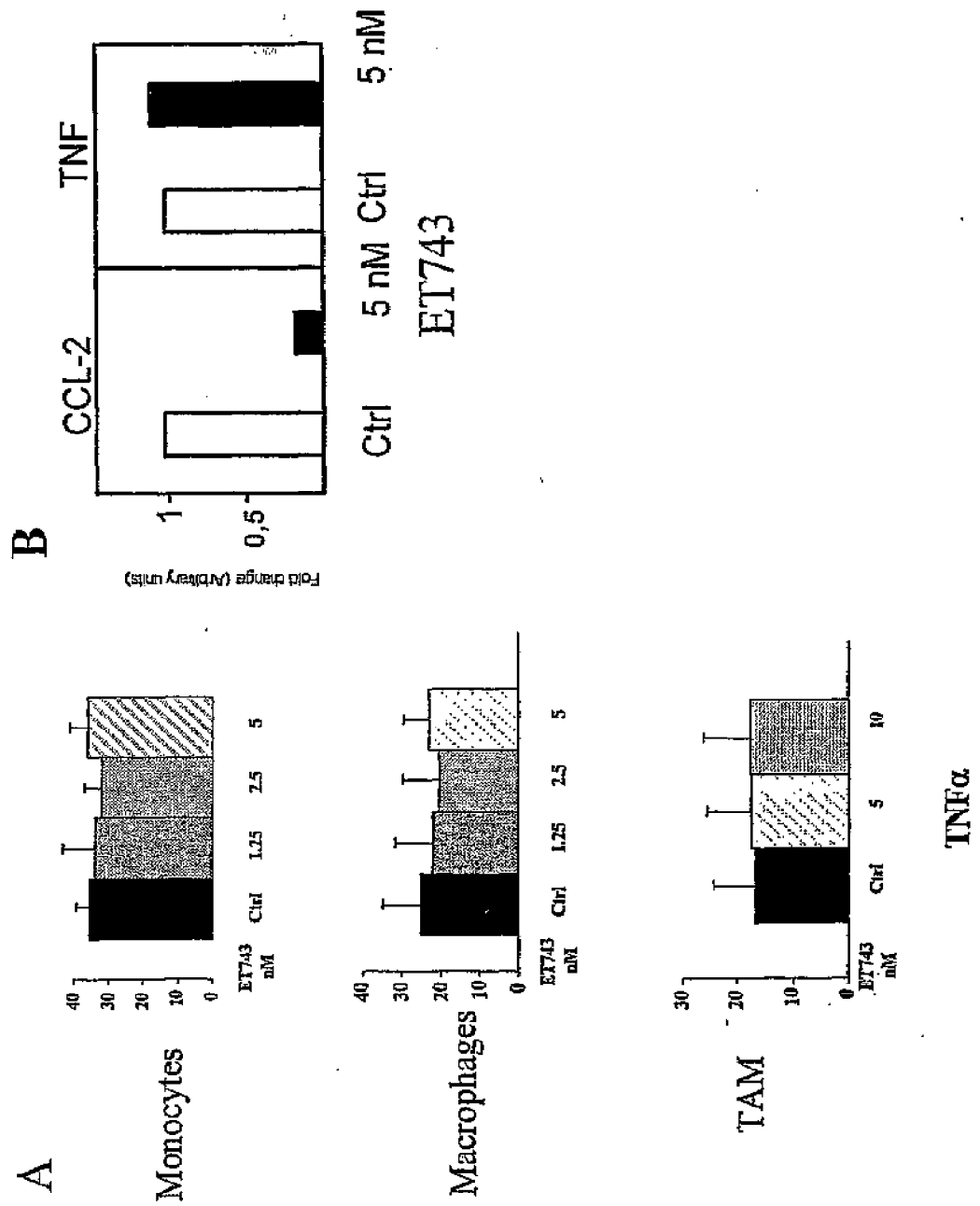


Figure 8



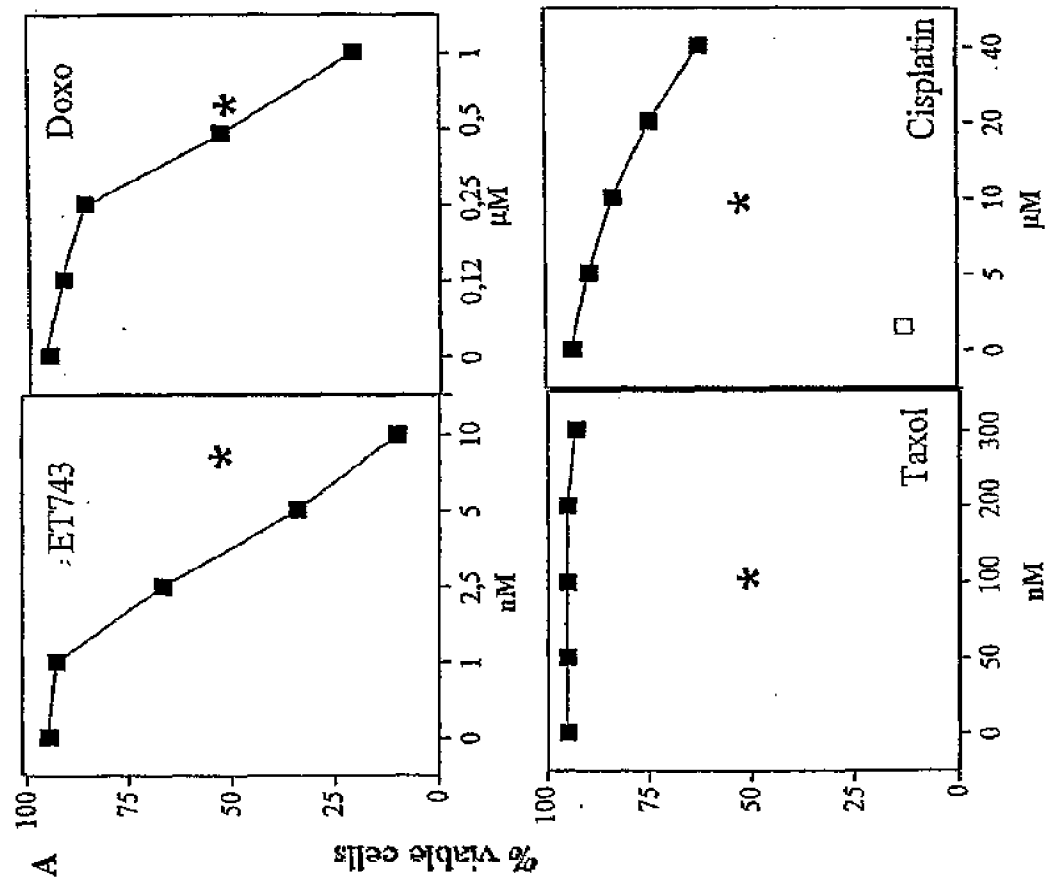


Figure 9A

Figure 9B

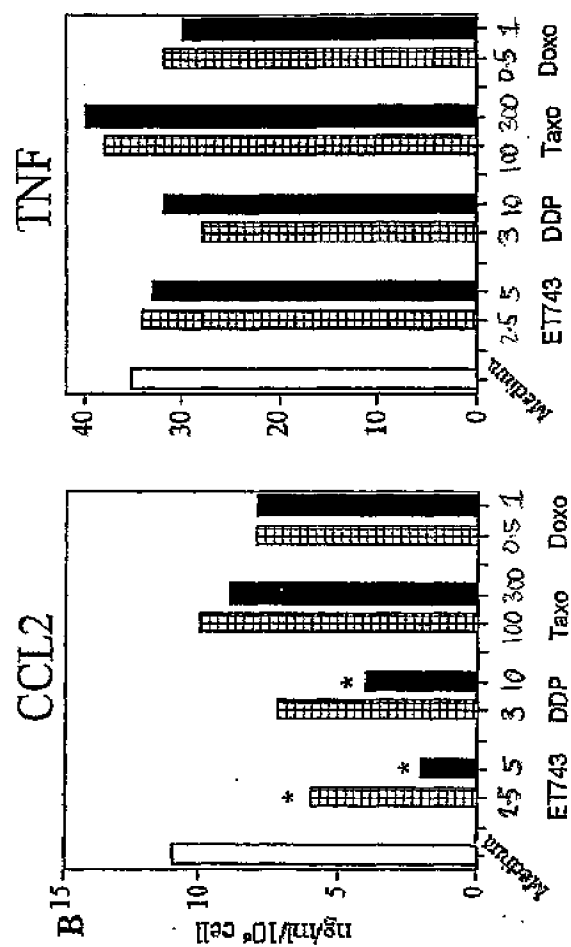
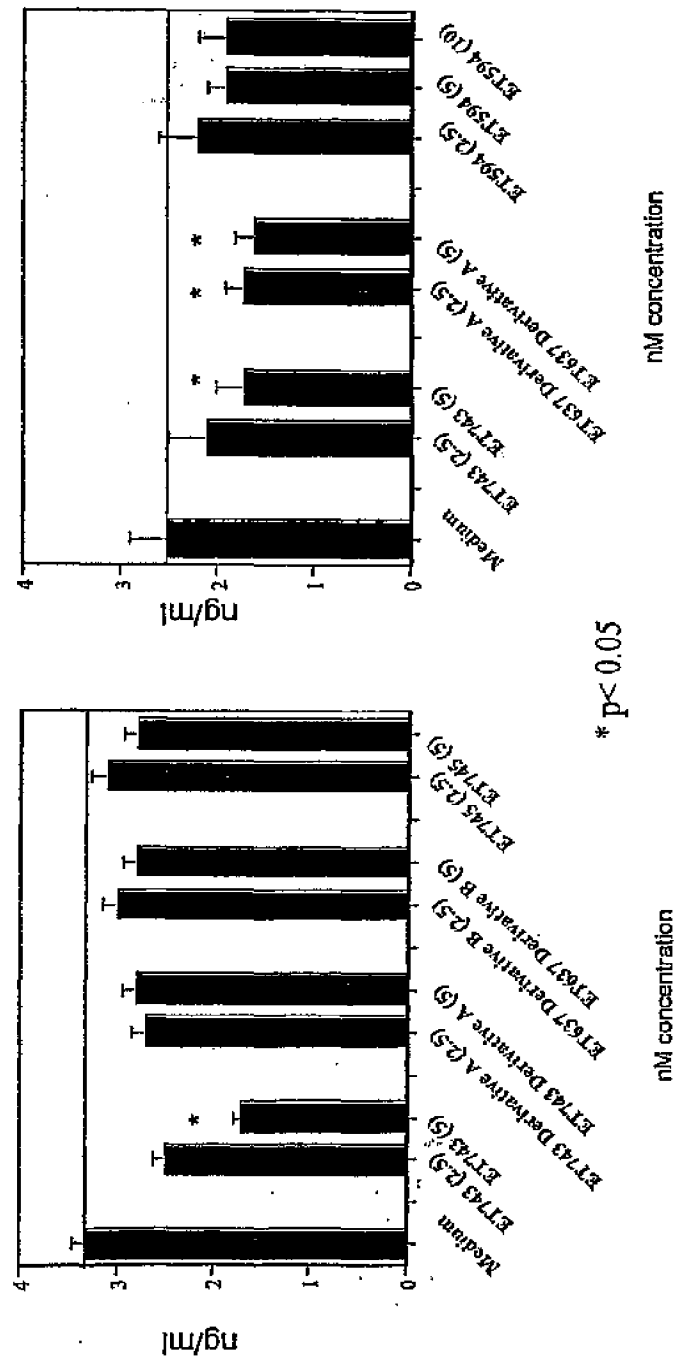


Figure 10



# INTERNATIONAL SEARCH REPORT

International application No  
PCT/GB2005/050164

A. CLASSIFICATION OF SUBJECT MATTER  
INV. A61P29/00 A61K31/498

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ALLAVENA, P.; SIGNORELLI, M.; CHIEPPA, M.; ERBA, E.; BIANCHI, G.; MARCHESI, F.; OMERO OLIMPIO, C.; BONARDI, C.; GARBI, A.; LISSONI: "Anti-inflammatory Properties of the Novel Antitumor Agent Yondelis (Trabectedin): Inhibition of Macrophage Differentiation and Cytokine Production" CANCER RESEARCH, 2005, pages 2964-2971, XP002382505 the whole document	1-13
X	WO 01/77115 A (PHARMA MAR, S.A; RUFFLES, GRAHAM, KEITH; FLORES, MARIA; FRANCESCH, AND) 18 October 2001 (2001-10-18) page 62 - page 77 page 79 - page 85 pages 87,89 pages 107-110	13
	-/-	



Further documents are listed in the continuation of Box C.



See patent family annex.

\* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

31 May 2006

Date of mailing of the international search report

16/06/2006

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Damiani, F

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/GB2005/050164

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03/066638 A (PHARMA MAR, S.A.U; MENCHACA, ROBERTO; MARTINEZ, VALENTIN; RODRIGUEZ, R) 14 August 2003 (2003-08-14) page 60 - page 64; compounds 11-15 page 65 - page 66; compounds ET-729, ET-770 page 69; compounds ET-594 page 72; compounds ET-745 page 73; compounds 18,19 page 81 - page 107; compounds 24-28, 30-33, 36-39, 41-46, 48, 50, 52, 54 page 118 - page 119; compounds 67,68 page 125 - page 130; compounds 73-77	13
X	US 5 256 663 A (RINEHART ET AL) 26 October 1993 (1993-10-26) column 1, line 25 - line 28	13
X	US 5 985 876 A (RINEHART ET AL) 16 November 1999 (1999-11-16) column 1 - column 3	13
X	US 2004/059112 A1 (RINEHART KENNETH L ET AL) 25 March 2004 (2004-03-25) the whole document	13
X	WO 00/69862 A (PHARMA MAR, S.A; RUFFLES, GRAHAM, KEITH; CUEVAS, CARMEN; PEREZ, MARTA;) 23 November 2000 (2000-11-23) page 77 - page 80; compounds 33-36	13
X	WO 03/008423 A (PHARMA MAR, S.A; MARTINEZ, VALENTIN; FLORES, MARIA; GALLEG0, PILAR; CU) 30 January 2003 (2003-01-30) page 33 - page 190; compounds 1-224	13
X	WO 99/51238 A (THE BOARD OF TRUSTEES OF THE UNIVERSITY OF ILLINOIS) 14 October 1999 (1999-10-14) page 2 page 4	13
X	ERBA, E.; CAVALLARO, E.; DAMIA, G.; MANTOVANI, R.; DI SILVIO, A.; DI FRANCESCO, A.M.; RICCARDI, R.; CUEVAS, C.; FAIRCLOTH, G.T.;D': "The unique Biological Features of the Marine Product Yondelis (ET-743, Trabectedin) are Shared by its Analog ET-637, which Lacks the C Ring" ONCOLOGY RESEARCH, vol. 14, 2004, pages 579-587, XP008064683 the whole document	13

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2005/050164

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0177115	A	18-10-2001	AT 299146 T	15-07-2005
			AU 784249 B2	23-02-2006
			AU 4672901 A	23-10-2001
			BG 107220 A	30-05-2003
			BR 0110024 A	18-02-2003
			CA 2406080 A1	18-10-2001
			CN 1436193 A	13-08-2003
			CZ 20023352 A3	14-05-2003
			DE 60111845 D1	11-08-2005
			DK 1280809 T3	07-11-2005
			EP 1280809 A1	05-02-2003
			HK 1049005 A1	27-01-2006
			HU 0300534 A2	28-07-2003
			JP 2003530402 T	14-10-2003
			NO 20024906 A	27-11-2002
			NZ 521550 A	29-10-2004
			PL 357574 A1	26-07-2004
			SK 14352002 A3	01-04-2003
			US 2003216397 A1	20-11-2003
WO 03066638	A	14-08-2003	AU 2003244453 A1	02-09-2003
			CA 2473175 A1	14-08-2003
			CN 1646539 A	27-07-2005
			EP 1472261 A2	03-11-2004
			JP 2005523275 T	04-08-2005
US 5256663	A	26-10-1993	NONE	
US 5985876	A	16-11-1999	NONE	
US 2004059112	A1	25-03-2004	NONE	
WO 0069862	A	23-11-2000	AT 283273 T	15-12-2004
			AU 775580 B2	05-08-2004
			AU 4597300 A	05-12-2000
			AU 5649301 A	26-11-2001
			AU 5649601 A	26-11-2001
			BG 107301 A	31-07-2003
			BR 0010559 A	02-07-2002
			BR 0110801 A	11-02-2003
			CA 2372058 A1	23-11-2000
			CN 1360588 A	24-07-2002
			CN 1440414 A	03-09-2003
			CN 1441805 A	10-09-2003
			CZ 20014102 A3	12-06-2002
			CZ 20023746 A3	16-04-2003
			CZ 20023751 A3	12-05-2004
			DE 60016209 D1	30-12-2004
			DE 60016209 T2	15-12-2005
			EP 1185536 A2	13-03-2002
			ES 2233367 T3	16-06-2005
			ES 2231486 T3	16-05-2005
			WO 0187894 A1	22-11-2001
			WO 0187895 A1	22-11-2001
			HU 0201188 A2	29-07-2002
			JP 2002544280 T	24-12-2002
			MX PA01011631 A	30-07-2002
			NO 20015547 A	14-01-2002



# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2005/050164

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0069862	A	NZ 515424 A	25-06-2004
		PL 353002 A1	22-09-2003
		PT 1185536 T	29-04-2005
		SK 16502001 A3	04-06-2002
		TR 200103273 T2	22-04-2002
		US 2004002602 A1	01-01-2004
WO 03008423	A 30-01-2003	CA 2453991 A1	30-01-2003
		EP 1406907 A1	14-04-2004
		JP 2004536132 T	02-12-2004
		MX PA04000506 A	23-07-2004
		US 2006106021 A1	18-05-2006
WO 9951238	A 14-10-1999	AU 758100 B2	13-03-2003
		AU 3471399 A	25-10-1999
		BG 104902 A	31-07-2001
		BR 9909488 A	26-12-2000
		CA 2327468 A1	14-10-1999
		CN 1304309 A	18-07-2001
		EP 1067933 A1	17-01-2001
		HU 0104273 A2	28-03-2002
		JP 2002510633 T	09-04-2002
		MX PA00009840 A	24-04-2002
		NO 20004978 A	05-12-2000
		NZ 507350 A	28-03-2003
		PL 343439 A1	13-08-2001
		RU 2217432 C2	27-11-2003
		SK 15012000 A3	06-08-2001
		TR 200002921 T2	21-02-2001